(3) *Plates.* Use 20 X 20 centimeter thin layer chromatography plates coated with silica gel 60F 254 or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* Mix petroleum ether (b.p. 60 to 80 ° C) and acetone in volumetric proportions of 100:30, respectively.

(2) *Spray solution.* Prepare a 1 percent solution of soluble starch in water (containing 0.01 percent mercuric iodide).

(c) *Preparation of spotting solutions*—(1) *Sample solution.* Prepare a solution of the rifabutin sample in 1:1 chloroform/methanol to contain 10 milligrams per milliliter.

(2) *Standard solution.* Prepare a solution of *N*-isobutylpiperidone standard in 1:1 chloroform/methanol to contain 1 milligram per milliliter. Transfer aliquots of 0.5, 1.0, 2.0, 5.0, and 10.0 milliliters into separate 100-milliliter volumetric flasks and dilute to volume with 1:1 chloroform/methanol. These solutions contain, respectively, the equivalent of 0.05, 0.1, 0.2, 0.5, and 1.0 percent of *N*-isobutylpiperidone.

(d) *Procedure.* Pour 100 milliliters of developing solvent into the glass trough on the bottom of the unlined chromatography tank. Cover and seal the tank. Allow it to equilibrate while the plate is being prepared. Prepare a plate as follows: on a line 2.0 centimeters from the base of the thin layer chromatography plate, and at intervals of 2.0 centimeters, apply 10 microliters of each of the standard solutions and the sample solution prepared as directed above. After the spots are thoroughly dry, place the plate into the trough in the bottom of the tank. Cover and tightly seal the tank, allow the solvent front to travel about 15 centimeters from the starting line and then remove the plate from the tank. Air dry the plate. Warm the iodine vapor chamber to vaporize the iodine crystals and place the dry plate in the iodine vapor chamber until the spots are visible (usually about 5 minutes). Remove the plate from the iodine vapor chamber and spray with 1 percent starch solution.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. *N*-isobutylpiperidone has an R_f value of about 0.3. Rifabutin has an R_f value of about 0.1. Compare the size and intensity of any *N*-isobutylpiperidone spots in the sample lane with the *N*-isobutylpiperidone spots in the standard lanes, and report the percentage of *N*-isobutylpiperidone in the sample.

[59 FR 40806, Aug. 10, 1994]

§ 436.370 Spectrophotometric identity test for rifabutin capsules.

(a) *Equipment.* A suitable spectrophotometer capable of recording the ultraviolet spectrum in the 200 to 400 nanometer range, using suitable quartz cells of 1 centimeter pathlength.

(b) *Preparation of working standard and sample solution*—(1) *Working standard solution.* Suspend approximately 200 milligrams of rifabutin working standard in 20 milliliters of methanol and sonicate for approximately 5 minutes. Filter the resulting solution through a suitable 0.5 micrometer filter. Transfer a 2-milliliter aliquot of the filtered solution to a 100-milliliter volumetric flask and fill to volume with methanol. Further dilute with methanol to obtain a solution containing 20 micrograms of rifabutin activity per milliliter.

(2) *Sample solution.* Empty and combine the contents of five capsules. Suspend a quantity of the capsule contents equivalent to 200 milligrams of rifabutin in 20 milliliters of methanol. Sonicate for about 5 minutes and then filter through an appropriate 0.5 micrometer filter. Transfer a 2-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with methanol. Further dilute with methanol to obtain a solution containing 20 micrograms of rifabutin activity per milliliter (estimated).

(c) *Procedure.* Using a suitable spectrophotometer equipped with 1.0 centimeter cells and methanol as the blank, determine the absorbance spectra of the working standard and sample solutions over the ultraviolet range of 250 to 300 nanometers.

(d) *Evaluation.* Compare the spectrum of the sample to that of the working standard. The identity of the rifabutin capsules is confirmed by quantitative comparison of the two spectra with an

absorbance maximum being observed at about 275 nanometers.

[59 FR 40807, Aug. 10, 1994]

Subpart G—Chemical Tests for Nonantibiotic Active Ingredients

§ 436.400 Thin layer chromatographic identity test for iodochlorhydroxyquin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 × 9 × 3.5 inches with a glass solvent trough on the bottom.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix benzene and methanol in volumetric proportions of 90:10.

(c) *Preparation of spotting solutions*—(1) *Sample solution.* Use the sample solution prepared as described in the section for the particular product to be tested.

(2) *Reference solution.* Prepare a solution containing 0.5 milligram of iodochlorhydroxyquin U.S.P. reference standard per milliliter in acetone.

(d) *Procedure.* Pour developing solvent into the glass trough on the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Spot a plate as follows: Apply approximately 10 microliters each of the sample solution and of the reference solution on a line 2.0 centimeters from the base of the silica gel plate and at intervals of not less than 2.0 centimeters. After all spots are thoroughly dry, place the silica gel plate directly into the glass trough of the chromatography tank. Cover and reseal the tank. Allow the solvent front to travel about 15 centimeters from the starting line, remove the plate from the tank, and allow to air dry. Examine under a strong source of ultraviolet light. The sample and standard are visible as dark spots.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values (0.55 to 0.60).

Subpart H—Tests for Specific Antibiotic Dosage Forms

§ 436.500 Penicillin in oil and wax.

(a) *Potency.* Proceed as directed in § 440.80a(b)(1) of this chapter except paragraph (b)(1)(ix) thereof and, in lieu of the directions in § 440.80a(b)(1)(iv) of this chapter prepare sample as follows: Liquefy the sample by warming, thoroughly mix, and withdraw 1.0 milliliter using a sterile syringe equipped with an 18-gauge needle. Transfer to a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the separatory funnel vigorously to bring about complete mixing of the material with the ether. Shake with a 25-milliliter portion of 1 percent phosphate buffer at pH 6.0. Remove the buffer layer and repeat the extraction with three 25-milliliter quantites of buffer. Combine the extracts and make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0. The sample may also be prepared by transferring aseptically 1.0 milliliter of the penicillin in oil and wax to a blending jar containing 100 milliliters of 1 percent phosphate buffer at pH 6.0. Using a high-speed blender, blend this mixture for 1 minute and then make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0. If the label represents the potency of the penicillin in oil and wax as 200,000 units per milliliter or less, it is satisfactory if it is 85 percent or more of the potency so represented; if represented as more than 200,000 units per milliliter, it is satisfactory if it is 90 percent or more of the potency so represented.

(b) *Sterility.* Proceed as directed in § 436.20, using the method described in paragraph (e)(2) of that section, except using medium B in lieu of medium A.

(c) *Moisture*—(1) *Reagents*—(i) *KarlFischer reagent.* Preserve the reagent in glass-stoppered bottles and use from an all glass automatic burette, protecting the solution from the moisture in the air.

(ii) *Water-methanol solution.* Use methanol containing approximately 1 mg. of water per milliliter. Store the solution in a glass bottle attached to an automatic burette and protect from moisture in the air at all times.